

# Resistance exercise and growth hormone as countermeasures for skeletal muscle atrophy in hindlimb-suspended rats

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**Linderman, Jon K., Kristin L. Gosselink, Frank W. Booth, Venkat R. Mukku, and Richard E. Grindeland.** Resistance exercise and growth hormone as countermeasures for skeletal muscle atrophy in hindlimb-suspended rats. *Am. J. Physiol. 267 (Regulatory Integrative Comp. Physiol. 36): R365–R371, 1994.—Unweighting of rat hindlimb muscles results in skeletal muscle atrophy, decreased protein synthesis, and reduced growth hormone (GH) secretion. Resistance exercise (ladder climbing) and GH treatment partially attenuate skeletal muscle atrophy in hypophysectomized hindlimb-suspended rats. It was hypothesized that a combination of multiple bouts of daily resistance exercise and GH ( $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ ) would prevent skeletal muscle atrophy in growing nonhypophysectomized hindlimb-suspended rats. Hindlimb suspension decreased the absolute (mg/pair) and relative (mg/100 g body wt) weights of the soleus, a slow-twitch plantar flexor, by 30 and 21%, respectively, and the absolute and relative weights of the gastrocnemius, a predominantly fast-twitch plantar flexor, by 20 and 11%, respectively ( $P < 0.05$ ). Exercise did not increase soleus mass but attenuated loss of relative wet weight in the gastrocnemius muscles of hindlimb-suspended rats ( $P < 0.05$ ). Hindlimb suspension decreased gastrocnemius myofibrillar protein content and synthesis (mg/day) by 26 and 64%, respectively ( $P < 0.05$ ). The combination of exercise and GH attenuated loss of gastrocnemius myofibrillar protein content and synthesis by 70 and 23%, respectively ( $P < 0.05$ ). Results of the present investigation indicate that a combination of GH and resistance exercise attenuates atrophy of unweighted fast-twitch skeletal muscles.*

protein synthesis; hindlimb suspension

suspended rats (12, 17), as well as the susceptibility of atrophic skeletal muscle to exercise-induced edema (18, 24), measurement of muscle wet weights may not accurately reflect the efficacy of exercise to attenuate muscle atrophy induced by exposure to hindlimb suspension. In contrast, skeletal muscle protein synthesis is affected to a greater degree by hindlimb suspension than muscle wet weight (26, 27), and the ~50% reduction in mixed and myofibrillar protein synthesis appears to play an important role in atrophy of the soleus muscle by hindlimb suspension.

Synthesis of myofibrillar proteins is a sensitive measure of the effects of both acute and chronic loading status of skeletal muscle and is regulated by the neuro-endocrine system (5). Myofibrillar protein synthesis is increased by muscle stretch (8), as well as acute and chronic muscle contractions (33), and decreases within 5 h of hindlimb suspension before a detectable change in muscle wet weight or protein content (26, 27). Muscle protein synthesis is impaired significantly in hypophysectomized rats (5), and rats exposed to spaceflight or hindlimb suspension exhibit decreased pituitary function and plasma bioactive growth hormone (GH) levels (9, 10). Thus reduced pituitary function during hindlimb suspension may reduce muscle protein synthesis, and administration of exogenous anabolic adjuvants may stimulate protein synthesis and attenuate muscle atrophy. However, anabolic adjuvants appear to have little effect on microgravity-induced muscle atrophy without the addition of muscle loading (16, 30, 31).

The purpose of the present study was to test two hypotheses. First, it was hypothesized that resistance exercise would attenuate decreased protein synthesis and muscle atrophy resulting from hindlimb suspension. Second, it was hypothesized that administration of growth hormone in combination with resistance exercise would be more effective than exercise alone in stimulating protein synthesis and attenuating skeletal muscle atrophy during hindlimb suspension.

## METHODS

*Animal care and use.* An animal care protocol was approved by the Institutional Animal Care and Use Committee of Ames Research Center in accord with the Ames Research Center Animal User's Guide (AHB 7180) and the guidelines of the National Institutes of Health. Male albino rats (Simonsen; Gilroy, CA) were housed singly in Plexiglas suspension cages (34) on a standard 12:12-h dark-light cycle in a room maintained at  $24 \pm 1^\circ\text{C}$  and given ground standard rat Chow and water ad libitum.

*Surgical procedures.* Rats weighing 225–250 g were anesthetized with an intraperitoneal injection of a ketamine (55

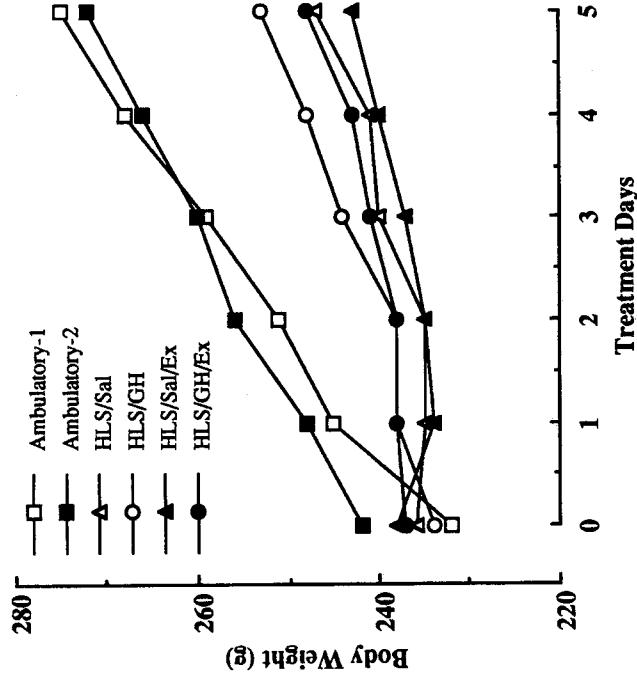


Fig. 1. Effect of 5 days of hindlimb suspension (HLS), recombinant human growth hormone (GH), and exercise (Ex) on mean body weight. Ambulatory-1, non-exercise-adapted controls; ambulatory-2, exercise-adapted ambulatory controls; Sal, saline-injected. SE bars are excluded for visual clarity. On average, SE was ~2%.

mg/kg body wt), xylazine (4 mg/kg), and acepromazine (0.75 mg/kg) cocktail, and carotid and jugular cannulas were implanted, as described previously (19).

**Experimental procedures.** On the third day of recovery from surgery, rats were allocated to one of five treatment groups: ambulatory control (ambulatory), hindlimb suspension with saline injection (HLS/Sal), hindlimb suspension with GH injection (HLS/GH), hindlimb suspension with saline injection and exercise (HLS/Sal/Ex), and hindlimb suspension with GH injections and exercise (HLS/GH/Ex). Animals were allocated, on the basis of their body weight, to treatment groups such that initial body weights were not different between groups (Fig. 1). Animals were tail-suspended for 5 days using the model described by Wronski and Morey-Holton (34). Recombinant human GH, equivalent to a replacement dose in hypophysectomized rats (1 mg/kg body wt; Ref. 11), was divided into two daily intraperitoneal injections (250 µg/ml 0.85% saline; Genentech, South San Francisco, CA) given at 0800 and 1600 h, immediately before exercise. Saline-injected rats received an equivalent volume of saline.

**Exercise training protocol.** For 3 days before and 3 days after surgery, rats were accustomed to ladder climbing on a 1-m grid (85° incline) with twice daily bouts of five repetitions with tail weights increased daily until the animals were climbing with an additional 50% of their body weight. During the 5-day period of hindlimb suspension, daily exercise training consisted of three daily bouts of 10 repetitions at 0800, 1200, and 1600 h, with an additional 50% of the animals' body weight attached to their tails (11). Tail weights were adjusted daily to changes in each animal's body weight. The nonexercised hindlimb-suspended and ambulatory rats were handled during each exercise training period, but care was taken to avoid contact with the hindlimbs. To account for possible effects of exercise acclimatization on body mass, muscle mass, and protein content, we utilized two control groups (ambulatory-1, ambulatory-2). One control group (ambulatory-1) was not accustomed to ladder climbing, and the other (ambula-

tory-2) was accustomed to ladder climbing as described above. No effect of exercise acclimatization was observed on body mass, muscle mass, or protein content. Therefore, the two groups were pooled for determination of protein synthesis.

**Measurement of protein synthesis.** After 5 days of treatment, HLS rats were infused in a suspended position for 5 h with [<sup>3</sup>H]leucine (Amersham, Arlington Heights, IL) at a rate of 1 mCi/h, in a Plexiglas cage similar to their suspension cage, while ambulatory animals were infused unrestrained. After anesthesia (pentobarbital sodium; 50 mg/kg body wt), muscles were dissected rapidly, trimmed of visible connective tissue, weighed, frozen in liquid nitrogen, and stored at -70°C. Mixed and myofibrillar protein fractions were isolated according to the procedures of Wong and Booth (33). Intracellular free amino acids were isolated from trichloroacetic acid supernatants by ion-exchange chromatography [Dowex-50 X8 (200-400 mesh, H<sup>+</sup> form); Bio-Rad, Richmond, CA]. Mixed and myofibrillar protein fractions were dissolved in 1 M NaOH; noncollagenous protein concentrations were determined with Pierce bicinchoninic acid reagent kits (Pierce, Rockford, IL) compared with bovine serum albumin standards in 1 M NaOH, and protein content was determined from the product of the protein concentration and muscle wet weight.

**Calculation of protein synthesis.** Rates of protein synthesis were estimated from the equation described by Garlick et al. (6) and programmed for use on a Macintosh computer (Think Pascal, Symantec) (26). The first-order rate constant for the rise in intracellular leucine was determined from the rise in plasma leucine specific activity in four rats, as described previously (32), and was found to be 73 half-lives per day. Protein synthesis (mg/day) was the product of the fractional rate of protein synthesis and protein content (1).

**Statistical analyses.** Data are presented as means ± SE. Body weights were compared using a two-way analysis of variance (ANOVA) with repeated measures (Super Anova; Abacus, Berkeley, CA). All other variables were compared using a one-way ANOVA. Where appropriate, differences between means were assessed with a Scheffé post hoc test. The 0.05 level of probability was chosen as significant for all analyses.

## RESULTS

**Body weight.** Body weight was significantly decreased by hindlimb suspension, increased by GH in hindlimb-suspended rats, and unaffected by exercise acclimation in ambulatory rats (Fig. 1). Ambulatory rats grew ~6 g/day, gaining an average of 30 g during the 5-day experimental period. Within 1 day of hindlimb suspension, body weights of all hindlimb-suspended rats were significantly less ( $P < 0.05$ ) than ambulatory-1 or ambulatory-2 animals and remained depressed throughout the 5 days of treatment. On the fourth day of hindlimb suspension, body weights of HLS/GH rats were 7 and 8 g greater than HLS/Sal and HLS/Sal/Ex rats, respectively ( $P < 0.05$ ). On the fifth day of hindlimb suspension, body weights of HLS/GH rats were 6 and 10 g greater than HLS/Sal and HLS/Sal/Ex animals, respectively ( $P < 0.05$ ).

**Muscle wet weight.** Absolute (mg/pair) and relative (mg/100 g) weights of the soleus were not different between ambulatory-1 and ambulatory-2 rats, indicating that exercise acclimatization before the 5-day experimental period had no effect on soleus wet weights in

Table 1. Effect of 5 days of hindlimb suspension, recombinant human GH, and exercise on plantar flexor muscle wet weight

	n	Soleus Weight		Gastrocnemius Weight		Plantaris Weight	
		Absolute		Relative		Absolute	
		Absolute	Relative	Absolute	Relative	Absolute	Relative
Ambulatory-1	8	239 ± 7	43 ± 1	3,068 ± 92	1,113 ± 22	608 ± 22	110 ± 3
HLS/Sal	10	167 ± 6*	34 ± 1*	2,442 ± 56*	990 ± 18*	485 ± 17*	98 ± 3*
HLS/GH	11	167 ± 4*	33 ± 1*	2,512 ± 39*	990 ± 16*	487 ± 17*	96 ± 2*
Ambulatory-2	8	227 ± 11	42 ± 2	2,979 ± 64	1,094 ± 17	594 ± 15	110 ± 2*
HLS/Sal/Ex	10	172 ± 5*	35 ± 1*	2,530 ± 66*	1,041 ± 19	500 ± 11*	103 ± 2
HLS/GH/Ex	10	177 ± 7*	36 ± 2*	2,588 ± 49*	1,045 ± 21	505 ± 13*	102 ± 3

Values are means ± SE; n = no. of rats/group. Absolute weights are in mg/muscle pair wet wt; relative weights are in mg muscle wet wt/100 g body wt. Ambulatory-1, non-exercise-adapted ambulatory controls; ambulatory-2, exercise-adapted ambulatory controls. Rats in various groups underwent indicated combinations of hindlimb suspension (HLS), saline (Sal) or growth hormone (GH) injection, and exercise (Ex).

\*Significantly different from appropriate ambulatory control ( $P < 0.05$ ).

control animals. Relative to ambulatory rats (ambulatory-1), absolute and relative wet weights of the soleus were decreased 30 and 21%, respectively, in HLS/Sal and HLS/GH animals ( $P < 0.05$ ; Table 1). Furthermore, the absolute and relative wet weights of the soleus in rats undergoing daily exercise alone (HLS/Sal/Ex) or in combination with GH (HLS/GH/Ex) remained less than in control (ambulatory-2) animals ( $P < 0.05$ ).

Prior exercise acclimatization also had no effect on the wet weights of either of the fast-twitch synergists, the gastrocnemius and plantaris muscles, in ambulatory rats (Table 1). Absolute and relative wet weights of the gastrocnemius and plantaris muscles of HLS/Sal rats were 20 and 11% less, respectively, than in ambulatory-1 animals ( $P < 0.05$ ). In addition, the absolute wet weight of the gastrocnemius and plantaris muscles in rats undergoing daily exercise alone (HLS/Sal/Ex) or in combination with GH (HLS/GH/Ex) remained less than in control (ambulatory-2) animals. However, the relative mass of the gastrocnemius and plantaris muscles was not different from ambulatory-2 rats and either HLS/Sal/Ex or HLS/GH/Ex animals. Collectively, the effect of hindlimb suspension on both absolute and relative wet weight in growing rats suggests that the soleus, gastrocnemius, and plantaris muscles underwent atrophy, defined as a decrease in absolute weight, and growth inhibition, defined as a decrease in relative wet weight. Furthermore, the positive effect of exercise on relative, but not absolute, wet weight in the gastrocnemius and plantaris muscles suggests that exercise prevented atrophy of fast-twitch muscles but did not attenuate growth inhibition.

**Protein.** Noncollagenous mixed protein concentration (mg/g) in the soleus muscle was unaffected by hindlimb suspension, exercise, or GH treatments (Table 2). How-

ever, mixed protein content (mg/muscle) was 22% less in HLS/Sal rats than in ambulatory animals ( $P < 0.05$ ). Soleus muscle mixed protein content in hindlimb-suspended rats was not affected by exercise or GH treatments. Furthermore, neither myofibrillar protein concentration nor content in the soleus was decreased by hindlimb suspension.

Gastrocnemius muscle protein concentration was unaffected by hindlimb suspension, exercise, or GH treatments (Table 3). Total protein content was 16% less in HLS/Sal rats than in ambulatory animals ( $P < 0.05$ ). No effect of exercise or GH treatments was observed on mixed protein content of the gastrocnemius in hindlimb-suspended rats. Similar to mixed protein, myofibrillar protein concentration in the gastrocnemius was unaffected by hindlimb suspension, exercise, or GH treatment (Table 2). Myofibrillar protein content was 26% less in HLS/Sal rats than ambulatory animals ( $P < 0.05$ ), and this loss of myofibrillar protein content was significantly attenuated by a combination of exercise and GH. Myofibrillar protein content was 25% greater in HLS/GH/Ex than in HLS/Sal rats ( $P < 0.05$ ), and no statistical difference existed between myofibrillar protein contents of HLS/GH/Ex and ambulatory animals.

**Protein synthesis.** Protein synthesis in the gastrocnemius muscle (Table 4) was affected relatively more by hindlimb suspension than either wet weight (Table 1) or protein content (Table 2). Fractional rates of mixed and myofibrillar protein synthesis (fraction/day) were 51 and 53% less, respectively, in HLS/Sal than in ambulatory rats ( $P < 0.05$ ). Similarly, the rates of mixed and myofibrillar protein synthesis (mg/day) were 60 and 64% less, respectively, in HLS/Sal than in ambulatory animals ( $P < 0.05$ ). Neither mixed nor myofibrillar protein synthesis was significantly affected by exercise

Table 2. Effect of 5 days of hindlimb suspension, recombinant human GH, and exercise on soleus muscle protein

	n	Mixed		Myofibrillar	
		mg/g muscle		mg/muscle pair	
		Absolute	Relative	Absolute	Relative
Ambulatory	8	142 ± 9	29 ± 2	64 ± 6	13 ± 1
HLS/Sal	7	134 ± 8	23 ± 1*	64 ± 11	11 ± 2
HLS/GH	9	142 ± 5	24 ± 1*	67 ± 7	11 ± 1
HLS/Sal/Ex	6	145 ± 7	24 ± 2*	68 ± 7	11 ± 1
HLS/GH/Ex	8	145 ± 13	24 ± 2*	65 ± 7	11 ± 1

Values are means ± SE; n = no. of rats/group. \* Significantly different from ambulatory control ( $P < 0.05$ ).

**Table 3. Effect of 5 days of hindlimb suspension, recombinant human GH, and exercise on gastrocnemius muscle protein**

	n	Mixed Protein		Myofibrillar Protein	
		mg/g muscle	mg/muscle pair	mg/g muscle	mg/muscle pair
Ambulatory	10	157 ± 3	234 ± 8	69 ± 2	103 ± 4
HLS/Sal	10	162 ± 9	196 ± 10*	63 ± 7	76 ± 9*
HLS/GH	11	156 ± 3	196 ± 6*	70 ± 5	88 ± 7*
HLS/Sal/Ex	10	162 ± 6	203 ± 7*	71 ± 3	89 ± 3*
HLS/GH/Ex	11	157 ± 6	206 ± 7*	74 ± 4	95 ± 4†

Values are means ± SE; n = no. of rats/group. \* Significantly different from ambulatory control ( $P < 0.05$ ); † significantly different from HLS/Sal rats ( $P < 0.05$ ).

or GH. However, the rates of mixed and myofibrillar protein synthesis (mg/day) in HLS/GH/Ex rats were 37 and 47% greater, respectively, than in HLS/Sal animals ( $P < 0.05$ ). Similarly, the fractional rates of mixed and myofibrillar protein synthesis (fraction/day) were 27 and 22% greater, respectively, in HLS/GH/Ex rats than in HLS/Sal animals; however, these increases were not significant ( $P = 0.10$ ). Insufficient soleus muscle tissue remained to perform synthesis measurements after determination of mixed and myofibrillar protein contents.

**Intracellular and protein-bound specific activities.** Rates of protein synthesis were determined from the specific activities of intracellular ( $S_i$ ) and protein-bound [ $^3\text{H}$ ]leucine ( $S_b$ ) (Table 5). In general, changes in protein synthesis (Table 4) are reflected by changes in  $S_b$ . Gastrocnemius  $S_i$  was 8–9 disintegrations · min $^{-1}$  · pmol $^{-1}$  and was unaffected by hindlimb suspension, exercise, or GH. In contrast, hindlimb suspension decreased mixed and myofibrillar  $S_b$  by 52 and 47%, respectively, which were similar in magnitude to reductions in protein synthetic rates (Table 4). Mixed protein  $S_b$  was 32% greater in HLS/GH/Ex rats than in HLS/Sal rats ( $P > 0.05$ ), which was similar in magnitude to the difference in mixed protein synthesis (mg/day) between these groups. In contrast, the increase in the rate of myofibrillar protein synthesis between HLS/GH/Ex and HLS/Sal rats is not reflected by an increase in myofibrillar  $S_b$ .

## DISCUSSION

Results from the present investigation confirm previously published results indicating that exposure to simulated microgravity (hindlimb suspension) induces decreases in hindlimb skeletal muscle wet weight, protein content, and protein synthesis. In addition, results

of the present investigation indicate that multiple bouts of daily resistance exercise (ladder climbing), with and without recombinant human GH treatment, attenuated loss of fast-twitch plantar flexor (gastrocnemius and plantaris) wet weight, but not in the slow-twitch plantar flexor (soleus). Only a combination of exercise and GH was capable of maintaining myofibrillar protein content in the gastrocnemius. Furthermore, results from the present investigation suggest that the increase in gastrocnemius myofibrillar protein synthesis in hindlimb-suspended rats with a combination of resistance exercise and GH was responsible, at least in part, for maintaining myofibrillar protein content in the gastrocnemius.

**Indexes of skeletal muscle atrophy.** Hindlimb suspension significantly reduced the absolute (mg/pair) and relative (mg/g body wt) wet weights of all three plantar flexors: soleus, gastrocnemius, and plantaris (Table 1). In addition, consistent with previous results (8, 23), the effect of hindlimb suspension on muscle wet weight was more pronounced in the predominantly slow-twitch soleus than in either of its predominantly fast-twitch synergists, the plantaris and gastrocnemius. Furthermore, similar to muscle wet weight, mixed and myofibrillar protein contents were decreased by hindlimb suspension (Tables 2 and 3).

Neither mixed nor myofibrillar protein concentrations (mg/g) were decreased by hindlimb suspension in the soleus or gastrocnemius muscles (Tables 2 and 3). However, mixed protein content (mg/muscle) was significantly decreased by hindlimb suspension in both soleus and gastrocnemius muscles, and myofibrillar protein content was decreased by hindlimb suspension in the gastrocnemius. Thomason et al. (26, 27) reported that hindlimb unweighting results in a preferential loss of myofibrillar protein. Consistent with these findings,

**Table 4. Effect of 5 days of hindlimb suspension, recombinant human GH, and exercise on gastrocnemius mixed and myofibrillar protein synthesis**

	n	Mixed Protein		Myofibrillar Protein	
		%/day	mg/day	T/day	mg/day
Ambulatory	10	10.6 ± 1.4	25.3 ± 4.0	10.7 ± 1.7	10.9 ± 1.9
HLS/Sal	10	5.2 ± 0.9*	10.1 ± 1.9*	5.0 ± 0.7*	3.6 ± 0.5*
HLS/GH	11	5.9 ± 0.5*	11.6 ± 0.9*	5.4 ± 0.8*	4.7 ± 0.8*
HLS/Sal/Ex	10	5.4 ± 0.3*	11.0 ± 0.8*	4.3 ± 0.5*	3.8 ± 0.5*
HLS/GH/Ex	11	6.6 ± 0.6*	13.8 ± 1.4**†	6.1 ± 1.0*	5.3 ± 0.6*†

Values are means ± SE; n = no. of rats/group (except n = 9 ambulatory controls for myofibrillar protein synthesis measurements).

\*Significantly different from ambulatory control ( $P < 0.05$ ); † significantly different from HLS/Sal rats ( $P < 0.05$ ).

**Table 5. Effect of 5 days of hindlimb suspension, recombinant human GH, and exercise on unbound and protein-bound leucine specific radioactivity in gastrocnemius muscle**

	n	S <sub>b</sub> , dpm/pmol	Mixed	S <sub>b</sub> , dpm/pmol	Myofibrillar
Ambulatory	8	8.6 ± 1.0	0.144 ± 0.010	0.131 ± 0.010	
HLS/Sal	7	8.7 ± 0.7	0.069 ± 0.007*	0.070 ± 0.006*	
HLS/GH	9	8.0 ± 0.4	0.084 ± 0.007*	0.075 ± 0.009*	
HLS/Sal/Ex	6	9.4 ± 0.9	0.088 ± 0.006*	0.070 ± 0.006*	
HLS/GH/Ex	8	8.4 ± 0.4	0.091 ± 0.008*	0.061 ± 0.009*	

Values are means ± SE; n = no. of rats/group. S<sub>b</sub>, specific radioactivity of mixed or myofibrillar protein-bound leucine; dpm, disintegrations per minute. \* Significantly different from ambulatory control ( $P < 0.05$ ).

results of the present investigation indicate that ~70% of the total decrease in gastrocnemius protein content was due to loss of myofibrillar protein. Furthermore, our results indicate that the synthesis of skeletal muscle protein was decreased relatively more by hindlimb suspension than protein content.

Hindlimb suspension decreased gastrocnemius mixed and myofibrillar protein synthetic rates (fraction/day) ~50% (Table 4). Consistent with our results, Goldspink et al. (8) reported that 5 days of hindlimb suspension decreased the fractional and total (mg/day) rates of gastrocnemius mixed protein synthesis by 46 and 56%, respectively. Similarly, 7 days of hindlimb suspension reportedly decreased the fractional rates of mixed and myofibrillar protein synthesis in the medial gastrocnemius by 35 and 54%, respectively (25). The effect of hindlimb suspension on mixed protein synthesis in the latter investigation (25) was less than that observed in the present investigation (Table 4) and less than that observed previously (8). These differences may be attributable to age and gender differences in rats utilized in the various studies.

**Attenuation of muscle atrophy.** In support of our first hypothesis daily bouts of resistance exercise (ladder climbing) attenuated loss of relative wet weight of fast-but not slow-twitch plantar flexors (Table 1). Neither absolute weight of the gastrocnemius and plantaris muscles nor body weight was increased by exercise in hindlimb-suspended rats. Attenuation of the decrease in relative wet weight of the gastrocnemius and plantaris muscles suggests that exercise attenuated muscle atrophy but had no effect on growth inhibition in hindlimb-suspended rats. Consistent with our observations, Herbert et al. (13) reported that four daily bouts of ladder climbing (8 repetitions) with an additional 75% of the rat's body weight attenuated loss of relative but not absolute mass of the medial gastrocnemius during 7 days of hindlimb suspension. As in the present investigation, these investigators reported that daily exercise training did not increase body weight in hindlimb-suspended rats.

In support of our second hypothesis the combination of daily resistance exercise and GH prevented nearly all of the loss of gastrocnemius myofibrillar protein content

during hindlimb suspension (Table 3). This latter observation suggests that the combination of multiple bouts of daily resistance exercise and GH stimulated myofibrillar growth during hindlimb suspension, a contention that is supported by results of other investigators (31).

However, in contrast to the results of Herbert and co-workers (13), we observed no effect of daily exercise on attenuation of soleus muscle atrophy.

Frequency of muscular activity rather than intensity appears to improve the efficacy of an exercise countermeasure in the soleus (2, 13, 18), which is a predominantly slow-twitch plantar flexor. Doubling the number of daily exercise bouts from 2 to 4 more than doubled the efficacy of ladder climbing as a countermeasure to soleus muscle atrophy in hindlimb-suspended rats (13). Thus the lack of an effect of ladder climbing on attenuation of soleus muscle atrophy in the present study may be due to differences in the frequency with which animals were exercised. Furthermore, results of past and present investigations indicate that GH has no effect in the unweighted soleus muscle (16).

Recombinant human GH (~4 mg·kg<sup>-1</sup>·day<sup>-1</sup>) reportedly did not attenuate loss of mass or prevent expression of fast-twitch characteristics in the soleus muscles of rats exposed to 4 days of spaceflight (16). Consistent with this latter observation, data from the present investigation indicate that GH treatment (1 mg·kg<sup>-1</sup>·day<sup>-1</sup>) alone had no effect on mass or myofibrillar protein content in the soleus or gastrocnemius muscles during 5 days of hindlimb suspension (Tables 3 and 4). Thus results of past (16) and present investigations (Tables 3 and 4) suggest that GH treatment alone does not prevent muscle atrophy during spaceflight or hindlimb suspension of up to 5 days. However, anabolic steroid treatment reportedly spared ~50% of the loss of mass and myofibrillar protein in the plantaris but not soleus during 6 wk of hindlimb suspension (30). Therefore, it is possible that GH treatment alone might be capable of attenuating loss of fast-twitch plantar flexor mass during a much more prolonged period of hindlimb suspension.

**Effects of exercise and GH on protein synthesis.** In support of our second hypothesis, the combination of exercise and GH increased gastrocnemius mixed and myofibrillar protein synthetic rates (mg/day) by 36 and 47%, respectively, in hindlimb-suspended rats (Table 4).

To our knowledge this is the first evidence that a therapeutic dose of exogenous GH in combination with resistance exercise is capable of stimulating skeletal muscle protein synthesis in intact (nonhypophysectomized) rats undergoing hindlimb suspension. Furthermore, it is our contention that the increase in gastrocnemius myofibrillar protein synthesis in hindlimb-suspended rats from the combination of GH and exercise was responsible, at least in part, for the maintenance of gastrocnemius myofibrillar protein content (Table 3).

Loss of gastrocnemius muscle protein in hindlimb-suspended rats is associated with a decrease in the rate of protein synthesis (Table 4), while attenuation of the microgravity-induced decrease in protein synthesis by passive stretch (8) or exercise plus GH is accompanied

by maintenance of skeletal muscle protein content (Table 3). Furthermore, the increase in protein synthesis (mg/day) in hindlimb-suspended rats due to either passive stretch (8) or exercise plus GH (Table 4) exceeds the magnitude of change in protein content (Table 3). It has been reported that casting the plantar flexors of hindlimb-suspended rats in a dorsiflexed position increases gastrocnemius mixed protein synthesis (mg/day) by 86% and mixed protein content 25% relative to contralateral limbs that were not stretched (8). In the present investigation daily resistance exercise, amounting to weight bearing for ~1% of the day, in combination with GH increased gastrocnemius myofibrillar protein synthesis (mg/day) by 47% (Table 4) and increased myofibrillar protein content by 25% (Table 3). Protein breakdown was not measured in the present investigation, and therefore the possibility that suppression of protein breakdown by the combination of exercise and GH was responsible for sparing myofibrillar protein cannot be excluded. However, neither GH nor muscle stretch reportedly decreases protein breakdown (5, 8).

**Summary.** Results obtained in hindlimb-suspended rats corroborate results of previous studies that indicate that simulated microgravity induces loss of skeletal muscle wet weight, which can be attenuated in fast-twitch plantar flexors by daily resistance exercise. In addition, we observed that a combination of exercise and exogenous recombinant human GH was capable of maintaining nearly all of the myofibrillar protein content in the gastrocnemius muscles of hindlimb-suspended rats. Furthermore, the combination of exercise and exogenous recombinant human GH increased myofibrillar protein synthesis in hindlimb-suspended rats. Finally, the apparent interactive effect of exercise and exogenous recombinant human GH on myofibrillar protein suggests that a minimal amount of daily resistance muscle loading exercise is required to potentiate the anabolic effect of GH on fast-twitch skeletal muscles during exposure to atrophic conditions.

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